

# Antibiogram and plasmid profile of *Escherichia coli* Isolates from Faeces of Cattle in Abeokuta, South West NIGERIA

B. K. Bello<sup>1</sup>, A. T. Balogun<sup>2</sup> and V.O. Oyetayo<sup>3</sup>

1 & 2 Lecturer Department of Biological sciences, College of Natural and Applied Sciences, Crescent University, P.M.B. 2104, Abeokuta, Ogun State, NIGERIA

Correspondence: E-mail- [tunlapa2k3@yahoo.com](mailto:tunlapa2k3@yahoo.com) , [ovofuta@yahoo.com](mailto:ovofuta@yahoo.com)

3 Lecturer Department of Microbiology, Federal University of Technology, Akure, NIGERIA

## ABSTRACT

Faecal samples collected from cattle in Abeokuta, south west Nigeria were analysed for *E. coli* counts, antibiotics susceptibility pattern of *E. coli* and the plasmid profile of *E. coli* isolates using standard techniques. *E. coli* were recovered from 30 samples representing 28.4% of 107 faecal samples of cattle collected. The faecal *E. coli* count ranges from  $1.1 \times 10^3$  to  $9.0 \times 10^3$  cfu/ml<sup>-1</sup>. The percentage prevalence of resistance to each antimicrobial agent tested were; Ampicillin, AMP (97%), COT (93%), Gentamicin, GEN (57%), Nalidixic acid, NAL (40%), Nitrofurantoin, NIT (93%), Cotrimoxazole, COL (20%), Streptomycin, STR (37%) and Tetracycline, TET (93%). The plasmid profile analysis of the *E. coli* isolates from cattle faeces shows a total of 18 different plasmid bands with molecular weight ranging from 2310 to 23632.83bp. The relatively high prevalence of resistance to antimicrobial agents exhibited by *E. coli* isolates from cattle faeces is an indication of the possible health risk that may occur as a result of the indiscriminate release of such faeces to the environment.

Key words: Antibiotics, resistance, *E. coli*, cattle, Faeces, plasmid

## INTRODUCTION

*Escherichia coli* (commonly abbreviated *E. coli*, named after Theodor Escherich) is a Gram-negative rod-shaped, non-sporulating bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls [1]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K<sub>2</sub> (2). *E. coli* are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for faecal contamination [3,4]. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of mutation, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology. However, the present research is focusing on the antibiotic sensitivity pattern and plasmid profile of *E. coli* isolates from cattle faeces sourced from Abeokuta South Western Nigeria.

## MATERIALS AND METHODS

The faecal samples used in this research were collected from 107 selected Cattle at Lafena Abattoir, Abeokuta North Local government area, Nigeria.

### Sample collection

A total of 107 Cattle were sampled. Faecal sample were collected from the intestine using sterile plastic bottle with tight cover. The samples were

collected by taking small portion of the solid part of the faeces from the intestine of the cattle using sterile hand gloves and immediately poured inside a sterile plastic container with tight cover. The samples were immediately transported to the laboratory for analysis.

### Isolation and identification of *Escherichia coli*

Isolation and identification of *Escherichia coli* were done using Eosine methylene blue agar (EMB). Individual colonies showing a green metallic sheen on EMB agar were further confirmed using biochemical tests [5,6]. The antibiotic sensitivity assay of *E. coli* isolates was determined using the disc diffusion method [7] and the plasmid extraction was done using TENS-Mini Prep method of analysis [8]. TENS is made up of the following components: Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%.

## RESULTS

### Percentage *E. coli* count obtained from selected cattle in abattoir Lafenwa, Abeokuta, South western Nigeria.

Fig. 1 show the percentage *E. coli* count obtained from selected cattle in Lafenwa abattoir, Abeokuta, South west Nigeria. A total of thirty (30) cattle representing 28.4% of 107 cattle shed *E. coli*, while no *E. coli* was found in faecal material collected from 77 cattle representing 71.96%.

### Antibiotics sensitivity pattern of *E. coli* isolates

All the 30 *E. coli* isolates obtained from cattle faeces collected from Lafenwa abattoir in Abeokuta North local government were tested against the selected antimicrobial agent (Fig. 2). All the 30 *E. coli* isolates showed multiple resistances to the eight

(8) antimicrobial agents used. The number and percentage resistance of *E. coli* isolates to each antimicrobial agent tested were: AMP (29) representing 97%; COT (28) representing 93%; GEN (17) representing 57%; NAL (12) representing 40%; NIT (28) representing 93%; COL (6) representing 20%; STR (11) representing 37% and representing TET (28) 93%. However, of the eight antimicrobial agents tested, resistance was highest to AMP (29) 97%. However, there is variation in the prevalence of the resistance of the *E. coli* isolates to the antimicrobial agent tested.

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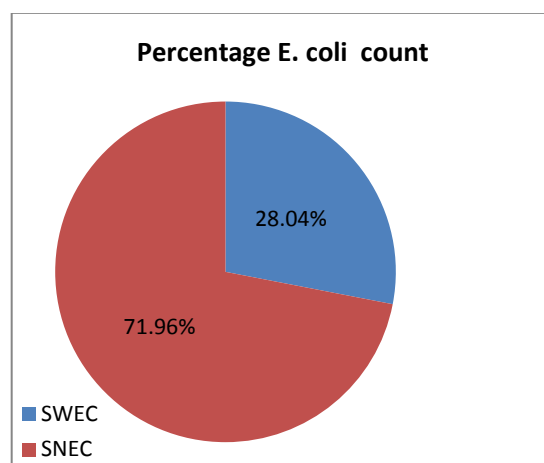
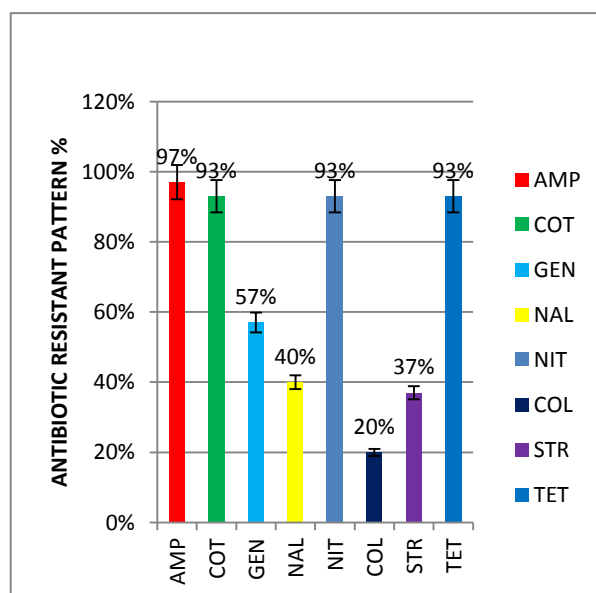


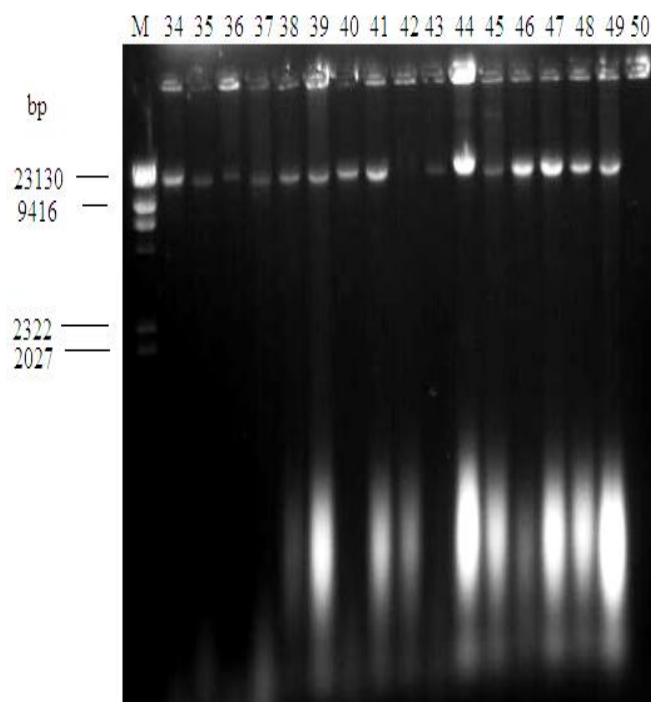
FIG. 1: Percentage *E. coli* Count. SWEC: sample with *E. coli*; SNEC: sample without *E. coli*



**Fig. 2: The percentage prevalence of resistance of the *E. coli* isolates to each antibiotic.** antibiotics used: AMPICILLIN (25µg), COTRIMOXAZOLE (20µg), GENTAMICIN (10µg), NALIDIXIC ACID (30µg), NITROFURANTOIN (200µg), COLISTIN (25µg), STREPTOMYCIN (25µg), and TETRACYCLINE (25µg).

**Table 1: Molecular weight of the *E. coli* plasmid band**

ISOLATES	MOLECULAR WEIGHT OF THE PLASMID (bp)
34.	23465.22
35.	23465.22
36.	21789.13
37.	23465.22
38.	23465.22
39.	23632.83
40.	23632.83
41.	23297.61
42.	23465.22
43.	23465.22
44.	8380.43, 13408.70, 21789.13
45.	10056.52, 21789.13
46.	21789.13
47.	21789.13
48.	21789.13
49.	21789.13
50.	23130



**Fig. 3: plasmid profile of *E. coli* isolates obtained from cattle analysed by 0.8% agarose gel electrophoresis after staining with ethidium bromide.**

Fig 3. Shows the plasmid profile of *E. coli* isolates obtained from cattle, analysed by 0.8% agarose gel electrophoresis after staining with ethidium bromide. Plasmid profile analysis of the *E. coli* isolates shows a total of 18 different plasmid bands. The molecular weight of these plasmid band ranges from 23130 to 23632.83bp and the highest number of plasmid bands (3 bands) was observed in isolate with serial number 44( Table 1).

## DISCUSSION

Cattle are one of the potential environmental sources of *E.coli* which may transfer transient bacteria into humans [9]. The result of this research revealed that most of the *E. coli* isolates were resistant to AMP (97%), COT, NIT, TET (93%) and GEN (57%). On the other hand, the isolates were highly sensitive to antibiotics such as COL (80%), STR (63%), and NAL (60%). The antibiotics resistance patterns of the isolates obtained in this study are in line with results from previous investigation [10,11]. Nalidixic acid was more potent in inhibiting the growth of *E. coli* isolates from cattle faeces. Nalidixic acid is a synthetic chemotherapeutic agent effective against gram-negative bacteria by binding to DNA gyrase enzyme (topoisomerase) thus, inhibits DNA duplication. It is mainly used in the treatment of urinary tract infections. The observed sensitivity of bacterial to colistin and streptomycin has been attributed to the restricted use of the drug [12]. Beta-lactam antibiotics inhibit the last step in the bacterial cell wall synthesis, while tetracycline blocks protein synthesis. The observed 97% resistance to AMP in this study is in line with the findings of [13,14]

The multidrug resistance *E. coli* are continuously increasingly, which has earlier been reflected by the findings of [15]. Such high incidence of multidrug resistance may presumably be due to indiscriminate use of antibiotics at the present time, which may eventually supercede the drug sensitive microorganisms from antibiotics saturated environment [16]. Most of the people are not well aware about the schedule of antibiotics course. Thus, a resistant strain emerges by genetic recombination against one or more antimicrobial agent [17]. The drug resistant bacteria can spread in the environment where man and animal acquire infection with bacteria carrying drug resistance plasmids [18]. *E. coli* strains range from highly pathogenic strains causing disease of the gastrointestinal urinary or central nervous system [19] to a virulent isolates which constitutes part of the non-pathogenic facultative flora of the human intestine [20]. The high prevalence of virulent *E. coli* strains as the primary causative agent of acute

diarrheal diseases has been reported extensively [21]. Over 500million cases of acute diarrhea have been reported to occur yearly in children age less than 5years, worldwide as a result of *E. coli* infection [22].

Plasmid profile analysis of 17 *E.coli* isolates by agarose gel electrophoresis showed a total of 18 different plasmid bands occurring in various combinations. [23] found 25 different plasmid bands in 63 *E. coli* isolates. The molecular weight of these bands ranges from 23130bp to 23632.83bp (Table 1). The distribution of different plasmids among these isolates appeared to have been at random. The plasmid profiles were compared with reference DNA molecular weight marker (Hind III digest of Lambda DNA). After electrophoresis, the band size was estimated by careful eyes estimation.

## CONCLUSION

Infections caused by strains of *E. coli* have become a significant public health problem worldwide. The major transmission route has been animal-to-person, waterborne and person-to-person. Cattle faeces has been recognized as the principal reservoir of *E. coli* in waterborne and foodborne *E. coli* outbreaks and sporadic infections. In this study, multiple antibiotic resistant strains of *E. coli* were isolated from faecal materials collected from cattle in Lafenwa abbatoir. This finding indicates that indiscriminate release of such faeces to the environment may lead to laden of the environment with *E. coli* with multiple antibiotic resistant characteristics. Hence, the continuous development of adequate preventive measures to diminish the spread of multi-resistant bacteria is needed for public health assurance.

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